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IN VITRO AND *IN VIVO* EFFECTS OF PDGF-BB DELIVERY STRATEGIES ON TENDON HEALING: A REVIEW.

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Abstract

To promote and support tendon healing, one viable strategy is the use or administration of growth factors at the wound/rupture site. Platelet derived growth factor-BB (PDGF-BB), together with other growth factors, is secreted by platelets after injury. PDGF-BB promotes mitogenesis and angiogenesis, which could accelerate tendon healing. Therefore, *in vitro* studies with PDGF-BB have been performed to determine its effect on tenocytes and tenoblasts. Moreover, accurate and sophisticated drug delivery devices, aiming for a sustained release of PDGF-BB, have been developed, either by using heparin-binding and fibrin-based matrices or different electrospinning techniques.

In this review, the structure and composition, as well as the healing process of tendons, are described. Part A deals with *in vitro* studies. They focus on the multiple effects evoked by PDGF-BB on the cellular level. Moreover, they address strategies for the sustained delivery of PDGF-BB. Part B focuses on animal models used to test different delivery strategies for PDGF-BB, in the context of tendon reconstruction. These studies showed that dosage and timing of PDGF-BB application are the most important factors for deciding which delivery device should be applied for a specific tendon laceration.

Keywords: PDGF-BB, tendon healing, heparin, electrospinning, biomechanics.

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Introduction

The healing of acutely injured tendons is a lengthy process, due to the inherent characteristics of these connective tissues. A poor vascular network and cells with low metabolic rate add to the poor intrinsic healing capacities andlimited regenerative potential of tendons. Often, the healing process is accompanied by development of a scar tissue next to properly regenerated tissue (Galatz *et al.*, 2015). The fibrous scar tissue has inferior properties compared to native tissue, resulting in functional and mechanical insufficiencies (Elliot and Giesen, 2013a). Biological therapies may help to overcome these problems. One approach, investigated to support tendon repair both *in vitro* and *in vivo*, is the application of growth factors (Bissell *et al.*, 2014; Hsu and Chang, 2004), including platelet-derived growth factor-BB (PDGF-BB) (Hee *et al.*, 2012).

PDGF-BB is part of the PDGF growth factor family, which includes four isoforms (A, B, C and D) (Andrae *et al.*, 2008). The PDGF-BB dimer – the mostly investigated isoform – is the only one that can bind to all three different surface PDGF receptors (PDGFRs) and trigger different signalling cascades, thus being called the universal isoform of PDGF. Compared to other growth factors, PDGF-BB has a well-established safety profile, approved by the Food and Drug Administration (FDA) (Borena *et al.*, 2015), and formulations supporting wound healing in foot ulcers, as well as, bone regeneration are on the market [Regranex Gel® (Smith&Nephew, London, UK) and GEM 21S® (Luitpold, Pharmaceuticals, Shirley, NY, USA)] (Howard *et al.*, 2014; Ma *et al.*, 2015). In chronic foot ulcers, topical application of rhPDGF led to a significant increase (by 43 %) in the incidence of complete wound closure and decrease in healing time (by 32 %) over placebo-controlled wound care, resulting in the FDA approval of Regranex Gel® (Wieman *et al.*, 1998). The effect of PDGF-BB in supporting osteogenic differentiation has been shown in an *in vitro* study with MG63 cells (Vahabi *et al.*, 2016). In addition, positive clinical results for GEM 21S® were reported in regenerative

periodontal surgery (Singh and Suresh, 2012). Moreover, platelet-rich plasma (PRP), blood plasma enriched with platelets, which release different growth factors (including PDGF-BB) upon activation by thrombin, has also been reported to be beneficial in a clinical setting [see reviews about PRP use in patellar tendinopathy (Jeong *et al.*, 2014) and PRP use in medical collateral ligament injuries (Andia and Maffulli, 2015)]. Injection of PRP has been shown to be superior to shock wave therapy when treating jumper's knee (patellar tendon or quadriceps tendon) (Vetrano *et al.*, 2013) and to reduce donor site morbidity in patellar tendons (de Almeida *et al.*, 2012). However, one big disadvantage of PRP is its variability in composition, due to different preparation protocols and patient differences, resulting in different effects with regard to growth factor composition and release (Marques *et al.*, 2015; Schaer *et al.*, 2015). Hence, mixed outcomes in clinical settings resulted after PRP application (Castillo *et al.*, 2011; de Vos *et al.*, 2010; Eppley *et al.*, 2004; Foster *et al.*, 2009; Kevy and Jacobson, 2004; Nikolidakis and Jansen, 2008). Therefore, a defined mixture of growth-factors or one single growth factor like PDGF-BB delivered in a controlled way present a good alternative for eliminating variability in treatment outcomes.

The functions of PDGF during tendon healing are manifold. After blood clot formation, platelets (thrombocytes) release a series of growth factors that interact with each other (Anitua *et al.*, 2007). PDGF attracts inflammatory cells, such as neutrophils and macrophages, responsible for the breakdown and phagocytosis of the debris (Deuel *et al.*, 1982; Inaba *et al.*, 1993; Tzeng *et al.*, 1985). Also, PDGF attracts tenocytes and fibroblasts that migrate to the wound site and start synthesising extracellular matrix components, including collagen (Banes *et al.*, 1995; Siegbahn *et al.*, 1990; Spindler *et al.*, 1995; Thomopoulos *et al.*, 2005).

When PDGF-BB is applied as a biological therapy, the time point of application and the dosage significantly impact its effectiveness. As the endogenous release of PDGF-BB is during the inflammatory and early proliferative phase (Chen *et al.*, 2008; Gulotta and Rodeo, 2009; Wuergler-Hauri *et al.*, 2007), it should be administered within the first two weeks after injury, at best using a delivery method that allows a controlled and sustained release. So far, clinical use of PDGF-BB in the tendon repair field has not been reported, with the main issue represented by its administration, *i.e.* providing a reliable delivery system that will allow for a sustained delivery of bioactive PDGF-BB at the injured site.

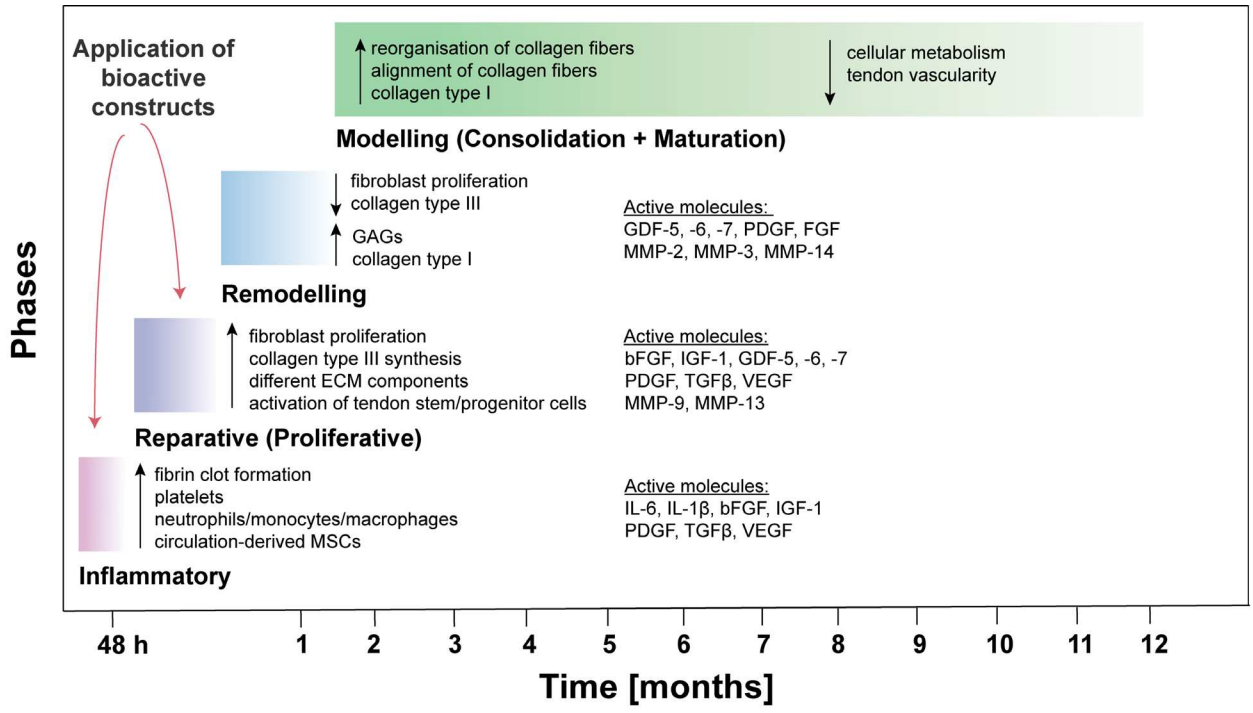

Fig. 1. General overview of the tendon healing process. After injury, several different, overlapping phases [inflammatory, reparative (proliferative), remodelling and later modelling phase] comprise the process of tendon healing, each one lasting for a shorter (hours) or longer (weeks) time period and marked with specific molecular, cellular and tissue changes. The length of each phase can differ in different species. The initial two phases (within 1-2 weeks post-injury) would be the most suitable time for the application of a construct delivering bioactive PDGF at the site of repair (Docheva *et al.*, 2015; Molloy *et al.*, 2003; Sharma and Maffulli, 2006).

Materials and Methods

Approximately 60 % of the papers mentioned in this review (total number of papers included in this review: 175) were found by a search in the Web of Knowledge/Web of Science/Google Scholar databases using the key words "tendon **AND** PDGF **OR** platelet derived growth factor"; "tenocyte **AND** PDGF **OR** platelet derived growth factor"; "PDGF **AND** sustained delivery"; "PDGF **AND** electrospinning" (the search was performed during the period of February-August 2016 and only literature written in English was reviewed). The remaining 40 % were cited in the references found within this search; with the exception of some very recent papers. The reviewed literature was published from 1978 to 2016. Original research papers, communications, review papers, as well as book chapters, were included. We focused on PDGF-BB administration to lacerated tendons, either by single bolus injections or released in a sustained manner from a delivery device. Moreover, full transections of tendons, partial lacerations and tendinopathy animal models, such as collagenase-induced tendinopathy, were included and the effects of PDGF-BB on the healing pattern were discussed. This review is divided into two main parts (A and B): *in vitro* and *in vivo* preclinical experiments.

Composition and healing of tendons

Tendons are constituted of fibres comprised of crosslinked collagen fibrils. Several different cell populations reside between chains of these long and parallel fibrils, including tenocytes (Lui, 2013), their precursor cells are called tenoblasts and tendon stem/progenitor cells (Bi *et al.*, 2007; Kannus, 2000). Tenocytes, spindle-shaped and elongated, are the most numerous cell population and they are responsible for the formation/turnover of extracellular matrix, assembly of early collagen fibres and facilitation of collagen network adaptation to external stimuli (Milz *et al.*, 2009). Tenoblasts, on the other hand, can vary in size and shape and are considered to be responsible for matrix (tissue) remodelling (Chuen *et al.*, 2004). Tendon stem/progenitor cells have been recently discovered and their capacity to differentiate into bone, cartilage or fat has been observed (Bi *et al.*, 2007), as well as, the expression of certain stem cells markers (Oct4 and SSEA4 among others) (Lui and Chan, 2011; Zhang and Wang, 2010b). The percentage of tendon stem/progenitor cells depends on age, species and type of tendon: younger specimens contain a higher percentage of tendon stem/progenitor cells (Zhou *et al.*, 2010) and the functional fitness of the cells is higher when compared to aged specimens (Spindler *et al.*, 1995).

The cell population, present within tendons, synthesises their necessary extracellular components, mainly collagens, glycosaminoglycans (GAGs), proteoglycans and elastin, and the composition varies slightly between tendons found in different locations of the body. Collagen fibres serve to maintain the tissue architecture, transmission and absorption of load and prevention of damage during mechanical stress (Pins *et al.*, 1997). Collagen type I (Col I) is the major extracellular matrix (ECM) constituent, roughly 65-80 % of the tendon dry mass (Kannus, 2000). Collagen type III (Col III) is the second most abundant collagen molecule and, although restricted to tendon sheets, it is the first collagen produced during tendon healing (Fig. 1) and is present in larger amounts in pathological tendons (Riley, 2004). Other collagen types in tendons include collagen type V, VI, XII, XIV and XV (Docheva *et al.*, 2015). In addition, elastic fibres composed of elastin and fibrillin are broadly distributed throughout tendons, with longitudinal localisation around cells and transversal localisation between collagen fascicles (Giusti and Pepe, 2016; Grant *et al.*, 2013; Kielty *et al.*, 2002). They allow tendon's extendibility and elasticity and are thought to play a role in the reestablishment of the crimp pattern of collagen fibres after tendon stretching (Butler *et al.*, 1978).

Even though mature tendons are characterised by low cellular density (~ 20 % of the total tissue volume; Nordin *et al.*, 2001), the cell population within the tendon is immediately affected upon tendon injury/damage. The healing process of tendons follows several phases, each characterised by different molecular elements and mechanisms (Fig. 1). Immediately after injury, a mix of cytokines and growth factors is released from the platelets and inflammatory cells,-such as macrophages, monocytes and neutrophils, attracted to the wound site (Fig. 2) and produce tumour necrosis factor (TNF) or growth factors involved in neovascularisation, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and PDGF (Chazaud, 2014; Lynch *et al.*, 1987). During healing, fibrin clot formation serves as a provisional scaffold, releasing a variety of growth factors, aiding the healing process. However, occasionally, this provisional scaffold is missing, as in the healing of anterior cruciate ligaments (ACL), and has been pointed out as a reason why the ACL does not have innate healing capacity (Murray *et al.*, 2000; Murray *et al.*, 2007; Murray and Fleming, 2013). The inflammatory phase takes place within the first hours after injury. It is followed by a proliferative, *i.e.* reparative, phase during which, fibroblasts, recruited from the tendon sheath and tendon, proliferate as a result of the mix of growth factors [transforming growth factor beta (TGFβ), FGF, Insulin-like growth factor 1 (IGF-1), PDGF and VEGF] produced at the wound site, and afterwards start synthesising ECM components, like collagen – predominantly type III – and proteoglycans. Angiogenesis, even though it may be thought as haphazard, is essential and beneficial, since lack of blood supply can impair the healing

process. Later, this transient capillary network has to retract so that the healing process can progress properly (Fenwick *et al.*, 2002).

In the next stage, remodelling of the tissue takes place by decrease of the cellular and vascular content and subsequent increase in deposition of collagen type I. Water content and glycosaminoglycan amounts stay larger in this phase (Oakes, 2008). In the modelling phase (consolidation + maturation), disorganised and randomly oriented collagen fibres are reorganised and healing tissue is reshaped and resized. During consolidation, the tissue changes from cellular to fibrous, synthesis of collagen type I still takes place and the collagen fibres become aligned in the direction of the stress (Hooley and Cohen, 1979). In the final stage, collagen fibril crosslinking is increased and the tissue changes gradually from fibrous to scar-like tendon tissue. The functionality after the healing is not the same as the one of a native tendon, due to structural aspects, *i.e.* alignment of collagen fibres, level of collagen cross-linking and natural crimp of collagen fibres differ from the native tendon (Connizzo *et al.*, 2013). In order to re-establish this aspect, proper tissue organisation, *i.e.* collagen fibre organisation, needs to be addressed and improved. Changes in elastic fibres during tendon healing have not been studied in detail yet, but initial evidence suggests that there is an increase in fibrillin-1 synthesis accompanied with a small increase in elastin production (Thakkar *et al.*, 2014). How this translates directly to the functional aspects of healed tendons is not clear yet. With regards to the cellular mechanism involved in tendon healing, it is believed that two mechanisms act together, intrinsic healing and extrinsic healing (Fenwick *et al.*, 2002; Kajikawa *et al.*, 2007). Initially, fibroblasts and inflammatory cells, from the tendon periphery and blood, are activated and migrate to the injury site, thus contributing to cell infiltration/adhesion formation and constituting the extrinsic mechanism (Beredjiklian, 2003). Later, the intrinsic mechanism takes place with cells from the endotenon being activated and migrating to the injury site, where they proliferate, synthesise ECM and play a role in its reorganisation (James *et al.*, 2008; Lin *et al.*, 2004). Indeed, one study has shown that the healing is a biphasic pattern (Kajikawa *et al.*, 2007).

The development of scar-like tendon tissue during healing leads to inferior mechanical, structural and biological properties, compared to non-injured tendons. For this reason, it has been proposed that application of bioactive constructs or injectable systems should aim at stimulating the intrinsic and suppressing the extrinsic healing mechanism to get improved restoration of the mechanical and functional properties of the healed tendons (Lomas

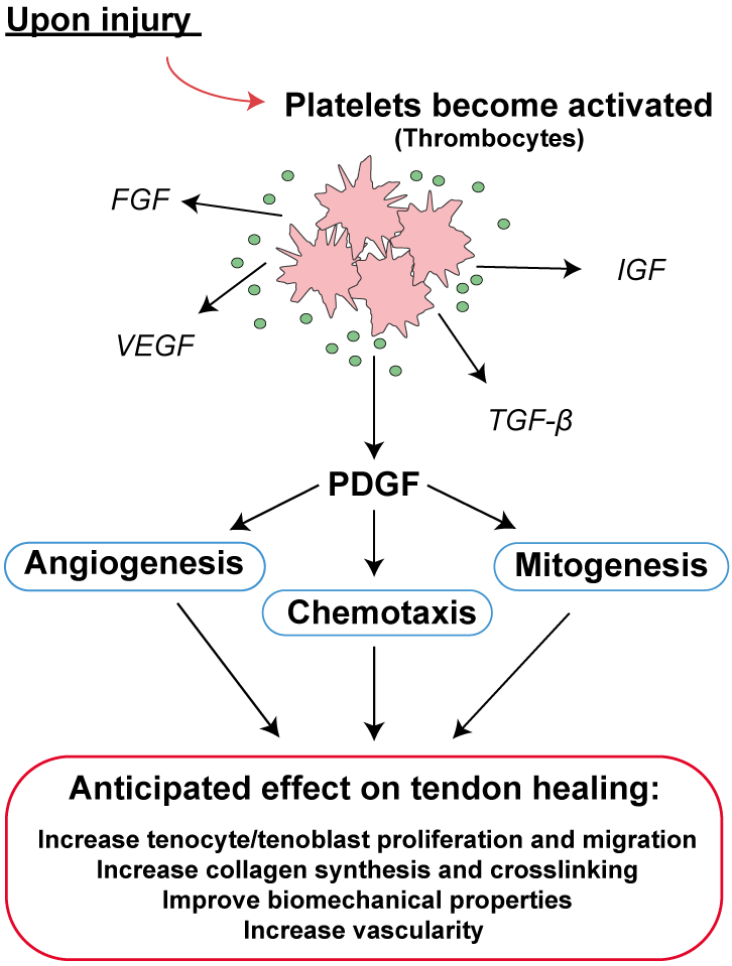

Fig. 2. Release and mechanism of action of PDGF after tendon injury. After an acute tendon injury, growth factors, such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and insulin like growth factor (IGF), are released from α-granules secreted by platelets at the wound site. PDGF upregulates VEGF and expression of integrins involved in smooth muscle cell migration, thus promoting angiogenesis. PDGF has chemotactic and mitogenic effects on neutrophils, macrophages and phagocytes, responsible for breakdown and cleaning of tissue debris, as well as, on tenocytes that enter the wound site to regenerate the damaged tissue. As a result, delivery of PDGF at the wound site can positively affect collagen deposition and crosslinking, biomechanical properties of a healing tendon, and also increase transient vascularisation by providing extrinsic factors for tendon repair.

et al., 2015; Tang, 2005). However, achieving good tendon regeneration, with proper tissue organisation and without any trans-differentiation of tenocytes into fibrocartilaginous or bone tissue (de Mos *et al.*, 2007; Zhang and Wang, 2010a), allowing proper tendon function and mechanical properties, still represents a major challenge when considering any biological strategy for tendon repair.

PDGF-BB is predominantly expressed during tendon healing

The pathways regulating normal tendon development are not completely understood, but it has been shown that FGF, TGFβ and growth differentiation factor (GDF) signalling regulate different aspects of tenogenesis. So far, PDGF involvement in tendon development has not been described. TGFβ (specifically TGFβ2/3) and FGF (specifically FGF4 and FGF8) signalling are shown to play a role in collagen expression/synthesis during development and in adult life (Brent and Tabin, 2004; Kuo *et al.*, 2008; Mikic *et al.*, 2006; Paxton *et al.*, 2012; Yun *et al.*, 2010). Tendon differentiation is mediated through the Smad signalling pathway of TGFβ (Lorda-Diez *et al.*, 2009; Pryce *et al.*, 2009). Disruption of FGF and TGFβ signalling leads to expression inhibition of the tendon associated transcription factor scleraxis (Scx) (Brent and Tabin, 2004; Brent *et al.*, 2005; Edom-Vovard *et al.*, 2002; Pryce *et al.*, 2009). Scx is a basic helix-loop-helix transcription factor, involved in regulating expression of other tenogenic markers such as tenascin-C, tenomodulin, Mohawk and type I collagen. It is expressed in tendon progenitor cells during embryonic development, as well as in mature tenocytes (Schweitzer *et al.*, 2001). Recently, the important role of Scx in tendon healing has been shown through the implantation of scleraxis-programmed tendon progenitors (hMSC-Scx), which enhanced the repair of a full-size rat Achilles tendon lesion (Hsieh *et al.*, 2016). Although some key players and pathways of tendon development are known, still many aspects remain unclear and need to be further investigated.

On the other hand, the growth factor profile during tendon healing differs from the one of tendon development (Glass *et al.*, 2014). Transforming growth factor beta 1 (TGFβ1) is present instead of TGFβ2/3, which in turn can activate IGF-1 secretion and, thus, have an impact on the functional recovery of the tendon (Chang *et al.*, 2000a; Klein *et al.*, 2002). PDGF and bone morphogenetic protein 12 (BMP-12) are moderately expressed overtime in the mid-substance of the tendon (Wuergler-Hauri *et al.*, 2007). Secreted by the platelets, FGF is also released at the wound site. The signalling pathways involved in development, TGFβ-Smad2/3 and FGF-ERK/MAPK , are also activated during the healing process (Nourissat *et al.*, 2015); however, it needs to be further elucidated as to how they interact or integrate during these two processes. Increase in mRNA levels of genes encoding for collagen, tenomodulin, tenascin-C and proteoglycans are observed during tendon healing, as well as upregulated expression of Scx and Mohawk right after injury (Juneja *et al.*, 2013; Scott *et al.*, 2011). The timing of all these cellular events is poorly understood. During the inflammatory phase and beginning of proliferative phase of the healing process, different isomers of PDGF are released from the platelets at the wound site (Andrae *et al.*, 2008).

PDGF-BB is a homodimer and one of the four isoforms (A, B, C and D) of the PDGF growth family. There are three cell-surface receptors through which PDGF signalling cascade takes place (PDGFRαα, PDGFRαβ and PDGFRββ) (Andrae *et al.*, 2008) **(**Fig. 3**A**). Once bound to its receptor, PDGF-BB initiates a signalling cascade and different cellular processes are affected through different signalling pathways (Fig. 3**B**). Some of the induced signalling pathways include Ras-MAPK, phosphoinositide 3-kinase (PI3K), phospholipase C gamma (PLCγ) and Janus kinase (JAK), which are involved in several cellular and developmental processes (Fig. 3**B**). For a comprehensive overview on the signalling pathways through which PDGF-BB elicits downstream cascades, refer to previous reviews on the topic (Andrae *et al.*, 2008; Heldin and Westermark, 1999; Tallquist and Kazlauskas, 2004). PDGFR can also interact with integrins, through the Na^+/H^+ exchanger regulatory factors (NHERFs) that link it to focal adhesion kinase and cytoskeleton (James *et al.*, 2004; Veevers-Lowe *et al.*, 2011). In turn, PDGFRs can be also affected by the ECM components (DeMali *et al.*, 1999; Veevers-Lowe *et al.*, 2011).

The tissue repair mechanisms induced upon PDGF-BB delivery are carried through its generic chemotactic, mitogenic and angiogenic properties, as well as its synergistic actions with other growth factors (Deuel *et al.*, 1991; Lynch *et al.*, 1987; Pierce *et al.*, 1991). PDGF has chemotactic and mitogenic effects on neutrophils, macrophages and phagocytes, responsible for breakdown and cleaning of tissue debris, as well as on tenocytes that enter the wound site to regenerate the damaged tissue. We believe that its ability to stimulate tenocyte and tenoblast proliferation, collagen production, collagen crosslinks and some new vessel formation, can aid the tendon healing process in the initial stages and lead to a better tissue organisation and subsequently improved biomechanical properties. The proliferative effect on tenocytes and tenoblasts supports the intrinsic healing mechanism, by attracting these cell populations from the endotenon, which in turn can synthesise and remodel the ECM. On the other hand, there is an evidence that the effect of PDGF-BB on elastin synthesis is inhibitory, where the MAPK/ERK signalling pathway acts in opposition to canonical TGFβ1 signalling (Sproul and Argraves, 2013). However, its mechanism and role in tendon healing are yet unknown. Since tendons are hypovascular, in the long run, this can be a drawback for the healing process, where some vascularisation can provide extrinsic factors for a better healing (Barrientos *et al.*,

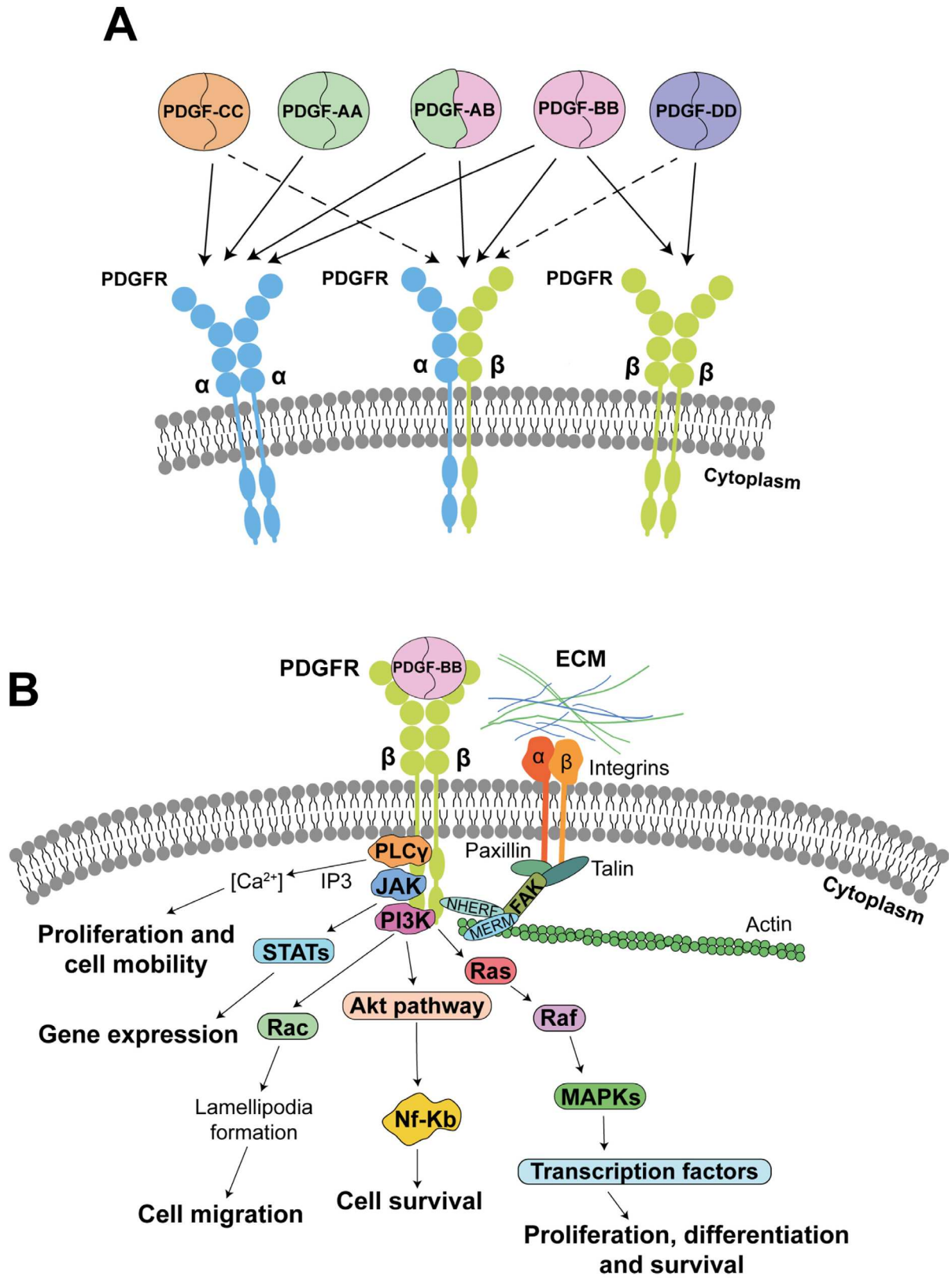

Fig. 3. PDGF binding and signalling pathways. (**A**) PDGF-PDGFR interactions, where each unit of the PDGF dimer interacts with one receptor subunit. The interactions shown have been determined in vitro; weak interactions or conflicting reports are represented with dashed lines. (**B**) Signalling pathways after PDGF-BB binding and interactions with the cytoskeleton and integrins. Simplified representation of the main players and actions where many other elements and processes, especially feedback mechanisms, have been omitted (based on Andrae *et al.*, 2008).

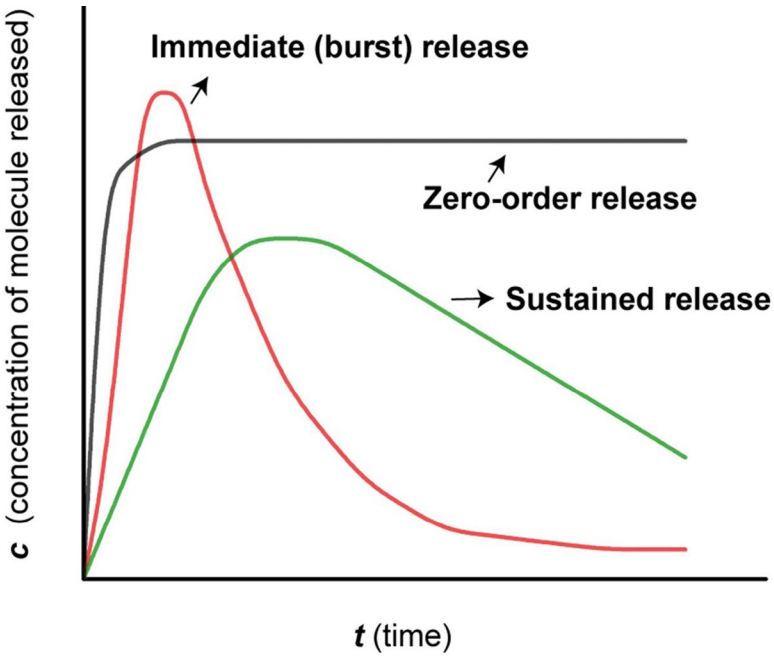

Fig. 4. Illustration of different molecules release profiles from polymeric delivery devices. Burst release is not desired, since almost the entire molecule of interest is released at once and very little or nothing is released in the subsequent time. Sustained release, which offers a more controllable rate of release, little by little, without having a big decrease in the rate of release is desired for most delivery devices and in the best case they approach first-order release kinetics. Zero-order kinetics allows for a constant rate of release of the molecule, independent on molecule concentration.

2014; Fenwick *et al.*, 2002). In this regard, application of PDGF-BB can prove beneficial, rather than VEGF, which has been shown to have deleterious effect on tendon healing, resulting in abundant hypoxia-inducible factor 1 (HIF-1)/VEGF-induced and matrix metallopeptidase 3 (MMP-3)-supported angiogenesis with inferior biomechanical properties of the tendons (Sahin *et al.*, 2012). The clinical efficacy of PDGF-BB (rhPDGF-BB) use in wound healing has been shown in several phase III studies, where its application is well tolerated and safe (Smiell *et al.*, 1999).

Part A: Effects of PDGF-BB delivery *in vitro*

Need for controlled and sustained release of PDGF-BB

Considering the healing process of acutely injured tendons, the correct timing of PDGF-BB administration is critical in determining the effectiveness of the growth factor therapy. Moreover, how PDGF-BB is delivered plays a role in whether the growth factor will be cleared right after administration or not. While PDGF-BB is ineffective when it is applied directly after injury [by injection (bolus)] or burst-released from a delivery device – which causes fast clearance of the growth actor at the wound site (Robinson and Talmadge, 2002) – a sustained release – allowing PDGF-BB presence at later time points, especially 7 d post-injury (Gulotta and Rodeo, 2009) – can lead to beneficial effects in terms of healing. Release refers to the process in which the molecule, *i.e.* growth factor of interest, migrates from the initial place within the polymeric system into the polymer's outer surface and then to the release medium (example: wound site) (Langer, 1990). The release is a process that is affected by different factors, including the structural characteristics of the delivery system, the method used to incorporate the molecule into the delivery system, the release environment *etc.*. Optimally, a delivery device should have a release profile approaching zero-order kinetics, meaning that the release of the molecule of interest takes place at a constant rate, independent of the molecule concentration involved in the process (Fig. 4). A burst release is characterised by an initial large release of the molecule from the system (within hours or days) without further changes within time. However, a sustained release offers a release of the molecule from the system in a controlled manner, little by little, at every time point, without having a large decrease in the rate of release. Most of the sustained release delivery devices result, at best, in first-order release kinetics. This can fit well in the case of PDGF-BB delivery device, where its presence would be desired 7 d post-injury, with a subsequent decrease and disappearance to avoid hypercellularity at the repair site. So far, several approaches have been tested for its delivery, which include heparin-based PDGF-BB immobilisation within different delivery devices or incorporation within polymeric scaffolds using different electrospinning techniques.

Heparin-based strategies for sustained PDGF-BB delivery

Heparin is a highly sulphated glycosaminoglycan, possessing moderate or strong binding affinity for several growth factors, including PDGF, FGF, TGFβ and VEGF (Guan *et al.*, 2004; Lyon *et al.*, 1997; Mangrulkar *et al.*, 1995). Investigated primarily with a focus to reduce thrombogenicity of materials in contact with blood, its use also spread to the development of drug delivery constructs. Through electrostatic interactions, the negatively-charged heparin molecules bind positively-charged growth factors, such as PDGF-BB, preventing quick diffusion

and retaining their bioactivity with protection from heat, pH and enzymatic degradation (Guan *et al.*, 2004). Heparin-based approaches for loading growth factors into constructs for tissue engineering applications have been used for different delivery systems including fibrin-based matrix (Thomopoulos *et al.*, 2007) and electrospun polymeric fibres (Lee *et al.*, 2012b).

Heparin-conjugated systems

These approaches utilise covalent immobilisation of heparin onto biomaterials by covalently binding it to proteins, such as collagen or albumin, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) chemistry (Hennink *et al.*, 1983; Wissink *et al.*, 2000; Wissink *et al.*, 2001). A demineralised bone matrix has been successfully crosslinked with heparin and loaded with PDGF-BB allowing for its sustained delivery and bioactivity retention (Sun *et al.*, 2009). Heparin conjugation with electrospun polymeric fibres with subsequent PDGF-BB loading (Fig. 5**A**) has been done in only a few studies using poly (ε-caprolactone) (PCL)/gelatin fibres (Lee *et al.*, 2012b), poly(L-lactide) fibres (plasma assisted heparin conjugation) (Cheng *et al.*, 2014) and PCL/gelatin electrospun fibres (Lee *et al.*, 2012a; Lee *et al.*, 2012b), where cellular bioactivity and cell infiltration were studied.

Fibrin-based delivery devices

The fibrin-based delivery system with heparin-immobilised PDGF-BB is the only one that has been explored for tendon regeneration application (Sakiyama-Elbert and Hubbell, 2000a; Sakiyama-Elbert and Hubbell, 2000b; Thomopoulos *et al.*, 2007). This delivery system is based on a bi-domain peptide that includes a factor XIIIa substrate, derived from α_2-plasmin inhibitor, at the N-terminus and a heparin-binding domain at the C-terminus. During coagulation, the bi-domain peptide is covalently crosslinked to the fibrin matrix by factor XIIIa. Heparin is immobilised electrostatically at the C-terminus and PDGF-BB is subsequently bound to heparin (Fig. 5**B**). In this system, compared to other heparin-based delivery systems, where heparin is covalently bound to the delivery construct, a non-covalent immobilisation is performed using primarily electrostatic interactions with the heparin-binding peptide. The release of PDGF-BB from the matrix can occur by dissociation from the matrix-bound heparin and subsequent diffusion, proteolytic degradation of the fibrin matrix and/or enzymatic degradation of heparin (Gelberman *et al.*, 2007).

Electrospinning – another approach for producing bioactive scaffolds delivering PDGF-BB

Electrospinning allows for production of scaffolds from different natural and synthetic polymers, fibrous and porous in structure, resembling the extracellular matrix (Rim *et al.*, 2013). Further modifications of the chemical, biological and mechanical properties of the scaffolds allow for advancements in applications. Methods for incorporation of bioactive molecules, like growth factors, within electrospun scaffolds, include physical adsorption (Kovacevic *et al.*, 2015) of biomolecules onto scaffolds, blend electrospinning, emulsion electrospinning and coaxial electrospinning (Fig. 6). While physical adsorption and blend electrospinning often result in burst release and can also cause growth factor denaturation, emulsion

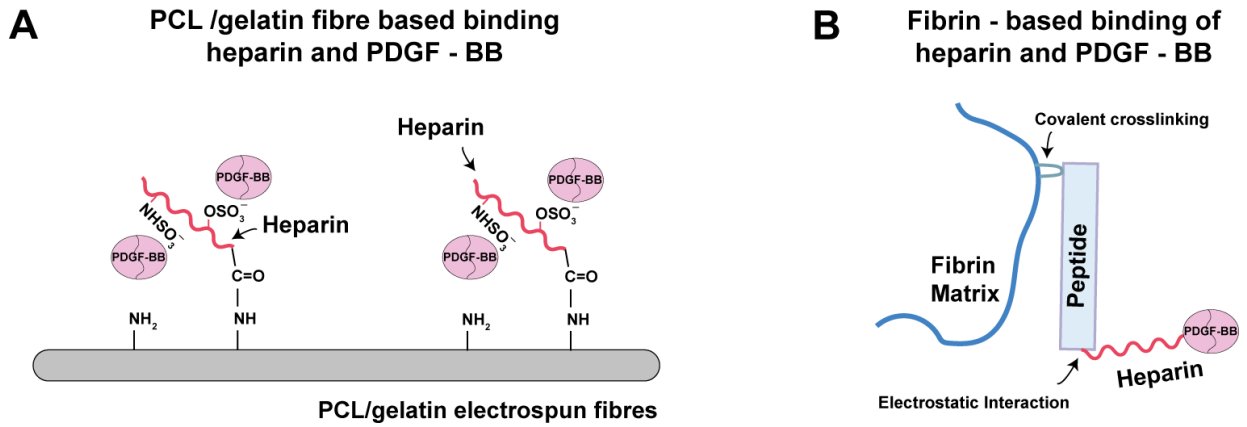

Fig. 5. Scheme of heparin-based delivery strategies for PDGF from different delivery matrices. (**A**) Scheme of heparin-conjugated electrospun PCL/gelatin fibres as a delivery device. The binding of PDGF-BB takes place in two steps. First, heparin is bound to the PCL/gelatin fibres through the formation of amide bonds, using EDC/NHS chemistry for activation of the carboxyl groups of heparin. Next, incubation with aqueous PDGF-BB allowed its immobilisation through electrostatic interactions with the heparin molecule. (**B**) Scheme of fibrin-based delivery system. During coagulation, a bi-domain peptide is covalently crosslinked to the fibrin matrix by factor XIIIa. Heparin is immobilised electrostatically at the C-terminus and PDGF-BB bound to heparin through electrostatic interactions is also immobilised within the carrier (Lee *et al.*, 2012a; Lee *et al.*, 2012b; Thomopoulos *et al.*, 2007).

and coaxial electrospinning are more promising approaches for a sustained PDGF-BB delivery.

In vitro sustained PDGF-BB delivery leads to similar biological responses to media supplemented PDGF-BB

Several characterisation studies throughout the years have been performed to determine the effects of PDGF-BB on tenocytes or tenoblasts in *in vitro* conditions, while its effects on tendon progenitor stem cells have not yet been tested. One of the main responses upon addition of PDGF-BB in a dose-dependent manner, either in serum free or complete culture medium, is the increase in proliferation of tenocytes (Table 1) (Banes *et al.*, 1995; Caliari and Harley, 2011; Costa *et al.*, 2006; Evrova *et al.*, 2016; Thomopoulos *et al.*, 2005; Wong *et al.*, 2003). Typically, the increase in cell proliferation has been assessed by metabolic activity assays or DNA synthesis quantification assays, while not exploring which pathway exactly led to the observed response. Maintaining tenocyte phenotype, while accelerating cell proliferation in the initial reparative phase, would be useful for aiding the initial tendon healing. Increase in collagen synthesis upon PDGF-BB addition has also been observed in a concentration-dependent manner, where the effect plateaued at a concentration of 20 ng/mL, which could be due to saturation of available cell receptors for certain growth factor (Costa *et al.*, 2006; Yoshikawa and Abrahamsson, 2001). Typically, 5-100 ng/mL PDGF-BB have been used as supplementation in *in vitro* experiments (Table 1), offering only an idea for the dosage that might be used in delivery devices or experiments *in vivo*.

Using the heparin-conjugation system, a demineralised bone matrix, as well as electrospun polymeric fibres successfully crosslinked with heparin and subsequently loaded with PDGF-BB,

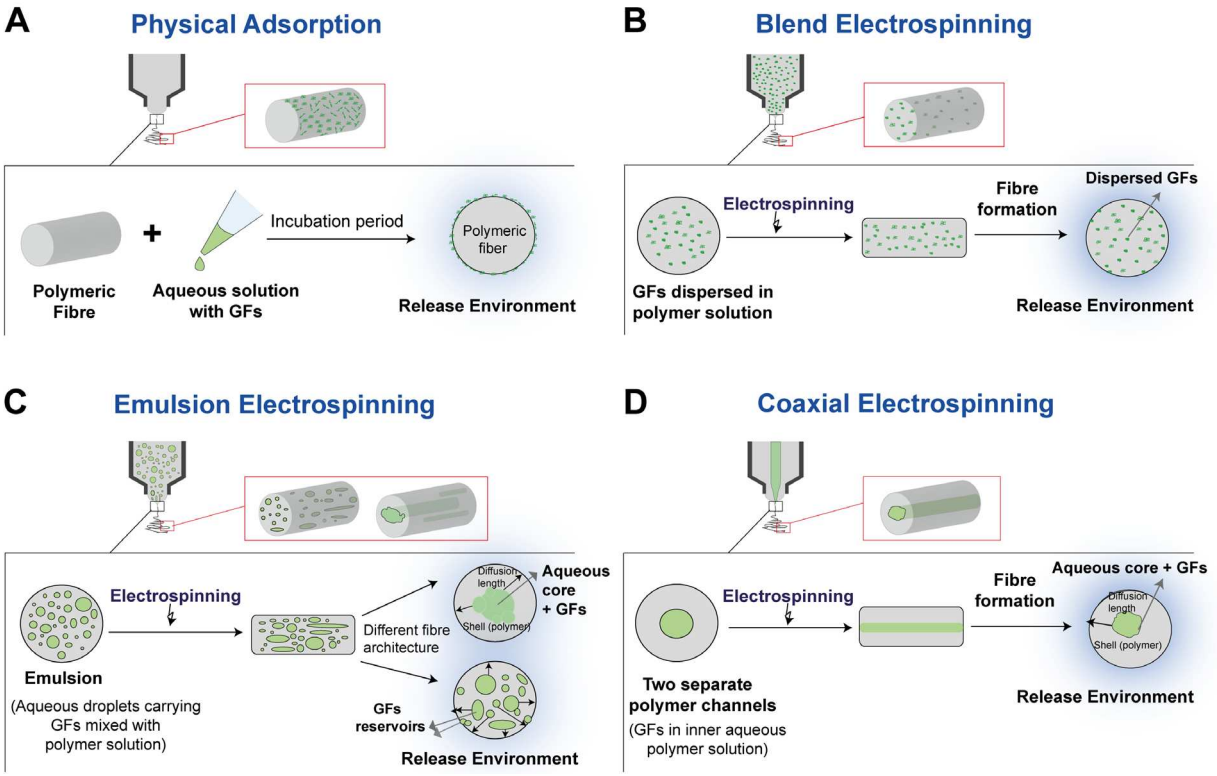

Fig. 6. Schematic overview of different electrospinning methods for growth factor (GF) incorporation within polymeric scaffolds. (**A**) Single electrospinning allows for standard polymeric fibres to be obtained from a polymer solution where afterwards growth factors (GFs) can be physically adsorbed onto. (**B**) Blend electrospinning allows GF incorporation into the polymeric fibres by simply dispersing the GF directly into the polymer solution and electrospinning this mix. However, this is not a preferred method, since biomolecules can be damaged/denatured by the presence of organic solvents in the polymer solution. Usually delivery devices obtained with this method exhibit a burst release of biomolecules from the fibres. (**C**) Emulsion electrospinning allows for GF incorporation initially in an aqueous solution, which forms the aqueous phase of the water-in-oil (w/o) emulsion, where the polymer solution is the oil phase. This method allows for better protection of the GF in the presence of organic solvents and results in devices with a burst or a sustained release profile. (**D**) Coaxial electrospinning allows core-shell formation within the polymeric fibres, where the aqueous phase, carrying the GF of interest, constitutes the core within the fibres. Being in the aqueous phase, the GF are protected within the polymer shell and usually their release is in a sustained manner, rather than a burst release, governed by GF diffusion from the core and pore formation within the polymer shell.

Table 1. Summary of *in vitro* tested conditions and cellular responses upon PDGF-BB administration.

In vitro model	PDGF concentration	Administration	Time point	Cellular response
Avian flexor tendon epitenon cells; internal fibroblasts (tenocytes) (P2–P4) (Banes *et al.*, 1995)	10, 50, 100 pM	Supplemented in serum free culture medium-1d	1 d	DNA synthesis ↑ in a dose-dependent manner in both cell populations. Mechanical stimulation had synergistic role on DNA synthesis
Canine intrasynovial flexor tendon fibroblasts (P2+) (Thomopoulos *et al.*, 2005)	10 ng/mL or 2-100 ng/mL in combination with bFGF	Supplemented in serum-free culture medium	1 d	Cell proliferation ↑ Total collagen synthesis ↑ Synergistic effect together with bFGF (within 5-40 ng/mL for DNA synthesis and 5-20 ng/mL for collagen synthesis)
Rabbit flexor tendon (synovial sheath, epitenon and endotenon) tenocytes (P4 or less) (Costa *et al.*, 2006)	1, 10 or 50 ng/mL	Supplemented in serum-free culture medium	3 d	Cell proliferation ↑ in a dose-dependent manner
Equine digital flexor tenocytes (P2-3) (Caliari and Harley, 2011)	10, 50 or 100 ng/mL	Supplemented in serum-free culture medium with tenocytes seeded on collagen-GAG scaffolds	1 d, 4 d, 7 d	Cell proliferation ↑ Cell metabolic activity ↑
Equine digital flexor tenocytes (P4) (Caliari *et al.*, 2014)	100 ng/mL	Supplemented in serum$^+$ culture medium	1 d	Tenocyte migration through collagen-GAG scaffold ↑
Rabbit Achilles tendon tenocytes (P1-P4) (Evrova *et al.*, 2016)	1-50 ng/mL or delivered by emulsion electrospun scaffolds	Supplemented in serum$^+$ and serum-free medium; PDGF-BB delivered from bioactive scaffolds	1 d, 3 d, 7 d, 14 d 1 d	Cell proliferation ↑ (serum free conditions); this effect was not significant in serum conditions. Cell proliferation of tenocytes on bioactive scaffolds ↑
Human patellar tendon tenocytes (P2-P4) (Wong *et al.*, 2003)	10 ng/mL	Supplemented in serum culture medium	2 d	PDGF-BB reversed the effects of dexamethasone which led to cell viability/proliferation ↓and collagen synthesis ↓
Human hamstring tenocytes (P3) (Qiu *et al.*, 2014)	5, 10, 50 ng/mL	Supplemented in serum-free culture medium	1 d, 7 d, 14 d	Cell proliferation ↑ Slight total collagen ↑ Tenomodulin, scleraxis, decorin expression ↓
Rabbit intrasynovial flexor tendon and extrasynovial peroneal tendon explants (Yoshikawa and Abrahamsson, 2001)	0.1-100 ng/mL	Supplemented in serum-free culture medium	4 d	Proteoglycan synthesis, collagen synthesis and cell proliferation ↑
Equine superficial digital flexor tendon explants (Haupt *et al.*, 2006)	1, 10, 50 or 100 ng/mL	Supplemented in culture medium with reduced serum (2 %)	6 d	Type I collagen gene expression ↑ Cell proliferation, GAG and total collagen content – n.a.

have been shown to successfully retain PDGF-BB bioactivity and allow for sustained delivery, compared to physically adsorbed PDGF-BB. The same delivery devices, with physically adsorbed PDGF-BB, exhibited a burst release of the growth factor within the first 3-4 d (Lee *et al.*, 2012a; Sun *et al.*, 2009).

Studies on tendon explants have shown results similar to cell cultures. Stimulated cell proliferation and collagen synthesis, upon PDGF-BB supplementation in the culture medium, were observed in intermediate and proximal intrasynovial flexor and extrasynovial peroneal tendon segments (Yoshikawa and Abrahamsson, 2001). On the other hand, Haupt *et al.* (2006), in a study on equine tendon explant, reported different results regarding the effect of PDGF-BB. No changes in morphology of the tendons, nor proliferative changes were detected upon addition of different concentrations of PDGF-BB. High concentrations led to increase in collagen type I gene expression and decrease in collagen type III gene expression, with no changes in the glycosaminoglycan content.

PDGF-BB was also shown to play a role in the regulation of different integrin receptors, namely alpha(v)beta3 and alpha5beta1 receptors. These specific integrins can be important in intrasynovial flexor tendon healing, since alpha5beta1 is involved in fibronectin deposition, as part of the provisional formed matrix. ECM remodelling might play a role in mechanotransduction (Regent *et al.*, 2011), while alpha(v)beta3 is involved in angiogenesis/revascularisation (Brooks *et al.*, 1994; Hodivala-Dilke, 2008). Semi-quantitative reverse transcription PCR showed that PDGF-BB increased expression of alpha(v) mRNA 3-fold, whereas alpha5 expression was increased 2-fold in intrasynovial flexor tendon cells (Harwood *et al.*, 1999).However, not much is known about effect of PDGF-BB on tendon specific markers, such as Scx or tenomodulin. Qiu *et al.* (2014), exploring different growth factor combinations for serum-free tenocyte expansion, observed a decrease in Scx, tenomodulin and decorin gene expression after 14 d, upon PDGF-BB supplementation. Tenocytes, cultured for 14 d in 50 ng/mL of PDGF, showed a similar expansion trend compared to tenocytes cultured in 10 % FBS (control group) and a slight increase in total collagen content and gene expression of type I collagen. Younesi *et al.* (2016) showed a decrease in Scx, tenomodulin and type I collagen gene expression in tenocytes cultured on collagen threads with immobilised PDGF-BB, in comparison to collagen threads only, but an increase in gene expression when compared to collagen gels.

Similar to media-supplemented PDGF-BB, different studies have addressed the bioactivity of PDGF-BB after being delivered by the system. These studies have assessed the effect of PDGF-BB on cell proliferation, *i.e.* increase in DNA content (Table 2).

So far, the most widely characterised delivery device for *in vitro* conditions has been a fibrin-based delivery system that allows immobilisation of heparin-binding growth factors, such as PDGF-BB, thus protecting them from degradation prior to release at the tendon injury site (Sakiyama-Elbert and Hubbell, 2000a; Sakiyama-Elbert *et al.*, 2008) (Table 2). This system, allowing for a sustained PDGF-BB delivery over a period of 10 d, has significant advantages compared to the bolus application of growth factors (growth factors are cleared within 48 h) (Robinson and Talmadge, 2002) or traditional synthetic polymeric delivery systems that can create acidic environment during degradation (Zisch *et al.*, 2003). Tested *in vitro* on canine tenocytes, fibrin matrices with PDGF-BB led to a significant increase in total DNA, compared to martices without PDGF-BB or matrices with PDGF-BB but without the delivery system. After 6 d in culture, collagen synthesis was enhanced to a greater extent by controlled delivery of PDGF-BB, rather than by PDGF-BB in fibrin matrices without delivery system, suggesting a need for its sustained delivery over time (Sakiyama-Elbert *et al.*, 2008). In a subsequent study, sustained delivery of PDGF-BB from the fibrin system resulted in downregulation of collagen (Col I and Col III) and lubrican gene expression at day 5. This suggests that downregulation of collagen genes by PDGF-BB may not necessarily translate into decreased production of collagen or that specific post-transcriptional events can play a role (Thomopoulos *et al.*, 2010a). Additionally, it has been suggested that PDGF-BB does not directly affect collagen synthesis, but rather that it is a potent chemoattractant for wound macrophages and fibroblasts, which may stimulate endogenous increase in TGFβ and, in turn, stimulate new collagen synthesis and enhancement in wound healing (Pierce *et al.*, 1989).

Due to the fact that the solely fibrin-based delivery system might not provide a surgically manageable construct for tendon repair, Manning *et al.* (2013) improved its structural integrity by layering it with an electrospun poly(lactic acid-co-glycolic acid) (PLGA) backbone and incorporating adipose-derived mesenchymal stem cells into the fibrin-based delivery system. The bioactivity of the delivered PDGF-BB was not directly assessed but, over a period of 14 d, the cell viability within the scaffolds was not affected.

Another delivery device tested for tendon healing application was an emulsion electrospun DegraPol® scaffold with incorporated PDGF-BB. When assessed *in vitro*, tenocytes showed increased proliferation as a result of released PDGF-BB in serum-free conditions or when directly seeded on bioactive scaffolds in serum conditions, thus showing PDGF-BB retained its bioactivity during the electrospinning process (Evrova *et al.*, 2016).

PDGF-BB delivery has been tested for other applications as well, where different delivery strategies were used (summarised in Table 2). In most studies, testing the bioactivity of incorporated and released PDGF-BB was done by looking at increase in cell proliferation, as the most pronounced biological

Table 2. Summary of devices used for delivery of PDGF-BB in tendon healing applications or other applications. key: ↑ = increase, n.a. = not affected, ↓ = decrease

Delivery Device	PDGF-BB loading	Release kinetics	*In vitro* model	Time point	Cellular response
Fibrin-based matrix with heparin bound PDGF-BB (Sakiyama-Elbert *et al.*, 2008)	0.125, 0.25 or 1.25 μg/mL per fibrin matrices (400 μL)	Sustained release-10 d; modulated by PDGF : heparin ratio (75-95 %) or PDGF-BB dose (55-95 %); the release of PDGF-BB was prolonged in the presence of cells	Canine flexor tendon fibroblasts	6 d	Cell proliferation ↑ Collagen production (0.125 μg/mL group) ↑
(Thomopoulos *et al.*, 2007)	40 ng per fibrin matrices (400 μL)	Sustained release – 10 d; burst release of physically adsorbed PDGF; slowest release – 1 : 10,000 PDGF-BB : heparin ratio (83 % by day 10)	Not assessed (only *in vivo*)	Not assessed (only *in vivo*)	Not Assessed (only *in vivo*)
(Thomopoulos *et al.*, 2009)	0.125, 0.25 or 1.25 μg/mL per fibrin matrices (400 μL)	Not assessed	Canine flexor tendon fibroblasts	5 d, 10 d	Cell proliferation ↑ Collagen I and III expression ↓ Lubricin and HAS2 expression –n.a. Decorin expression ↑
Fibrin-based matrix with heparin bound PDGF-BB + PLGA (Manning *et al.*, 2013)	50 ng per scaffold	Sustained release-9 d (71 % release by day 9)	Canine adipose derived mesenchymal stem cells	1 d, 3 d, 7 d, 11 d, 14 d	Cell viability – n.a.
DegraPol® emulsion electrospun scaffolds (Evrova *et al.*, 2016)	8 μg to 5 g of polymer solution	Sustained release-30 d (1.2 % release calculated on theoretical loading by day 30; 160 ng/mg of scaffold within 30 d)	Rabbit Achilles tendon fibroblasts	1 d	DNA synthesis ↑
PEO/PCL emulsion electrospun scaffolds (Briggs and Arinzeh, 2013)	1 μg or 10 μg (0.2 % w/w)	Emulsified addition in the presence of Span80 led to a sustained release up to 96 h; with 10 μg loading, cumulative release of 97 % within 31 d	Human mesenchymal stem cells	4 d, 11 d	Cell number ↑ ALP activity ↑
PEO/PCL + HA/TCP scaffolds (Briggs *et al.*, 2015)	10 μg (0.2 % w/w)	Sustained release up to 96 h		7 d, 21 d 4d, 11d	Cell number at 7 d ↓ Cell number at 21 d ↑ Osteocalcin production ↑ AP activity ↑
PCL coaxially electrospun scaffolds (Liao *et al.*, 2006)	20 μg per scaffold	Sustained release over 40 days (95 % release within 40d)	NIH 3T3	1 d, 7 d, 14 d, 20 d	Cell proliferation ↑
Demineralized bone matrix with crosslinked heparin (Sun *et al.*, 2009)	Incubated with 55, 110 or 220 μg/mL	Not assessed	Human fibroblasts	4 d	Cell proliferation ↑
PCL/gelatin with or without crosslinked heparin (Lee *et al.*, 2012)	100 ng per 1 × 1 cm^2 scaffold sample	Without heparin-5 d burst release; With heparin-sustained release over 20 d	Human smooth muscle cells	3 d, 7 d 1, 2 4 weeks	Cell proliferation ↑ DNA content ↑ Cell infiltration ↑

outcome after PDGF-BB supplementation (Table 2). Significant increase in cell proliferation upon PDGF-BB delivery, using different systems, was usually observed after 4 or 7 d, similar to PDGF-BB supplemented directly in the culture medium (Table 1). When DNA synthesis was studied, a short time point of 24 h was used for the delivery devices or media supplementation and a significant increase in DNA synthesis after PDGF-BB stimulation was observed in both cases (Table 1,2). However, besides the well-established proliferative effects, more systematic studies on the effect of PDGF-BB on some of the tendon specific markers in tenocytes and tendon stem cells are needed in order to obtain more conclusive insight about its possible impact in tendon healing.

The loaded amounts of PDGF into the delivery devices explored (Table 2) are generally larger than the amounts supplemented directly in culture medium (Table 1). However, usually 100 % loading efficiency within the device is not achieved and beside the sustained delivery they offer, still in some systems, under *in vitro* conditions, large amounts of encapsulated PDGF-BB are not released. Taking this into account, the amount of released PDGF-BB over time could be smaller or comparable to the media supplemented one, which, on the other hand, can drastically differ once the delivery device is used in *in vivo* conditions.

PDGF-BB delivery device design: easy handling, surgery compatibility and sustained release kinetics are important for successful application in tendon repair

Most strategies explored for PDGF-BB delivery experienced a sustained release of PDGF-BB over a period of several days (Table 2). A sustained release of growth factor was achieved using heparin immobilisation on PCL/gelatine scaffolds, where physical adsorption of PDGF-BB on the scaffolds resulted in a burst release within the first 3-4 d (Lee *et al.*, 2012b).

The release kinetics of PDGF-BB from the fibrin-matrix-based delivery system can be modulated by different molar ratios of PDGF-BB to heparin, different amounts of PDGF-BB loading and also different gel size (Sakiyama-Elbert *et al.*, 2008). In *in vitro* conditions, decreasing the molar ratio of PDGF-BB to heparin, from 1 : 10 to 1 : 10,000, led to significantly more sustained delivery of PDGF-BB within 10 d (Sakiyama-Elbert *et al.*, 2008; Thomopoulos *et al.*, 2007). Three different doses of PDGF-BB loading were evaluated (0.125, 0.25 and 1.25 μg/mL), where increase in the amount loaded led to more sustained release. Varying the fibrin matrix size did not have a major effect on the release rate of PDGF-BB and its passive release should correlate between different matrix volumes (Sakiyama-Elbert *et al.*, 2008). The release of PDGF-BB from the fibrin-based matrices was tested in the presence of cells and it was observed that its release is in a dose-dependent manner and similar to the *in vitro* passive release (Sakiyama-Elbert *et al.*, 2008).

The explored electrospun scaffolds, produced with either emulsion or coaxial electrospinning, also allow for sustained PDGF-BB delivery (Table 2). PDGF-BB has been incorporated within PCL and PCL/poly(ethylene oxide) (PEO) electrospun scaffolds, intended for bone tissue engineering applications (Briggs and Arinzeh, 2013; Briggs *et al.*, 2015). These types of scaffolds allow for sustained delivery of PDGF-BB over a period of 4 d, but without complete release of the growth factor from the polymeric scaffolds, while a fraction of it is likely to be bound to the scaffold in a bioactive form (Briggs *et al.*, 2015). Recently, DegraPol®, an elastic polyester urethane, block copolymer, has been studied as a delivery device for PDGF-BB. It allowed for the successful incorporation and sustained release of PDGF-BB within a period of 30 d. However, similar to the PCL and PCL/PEO scaffolds, a large amount of growth factor was still inside the scaffold or strongly bound to the surface and, therefore, not released (Evrova *et al.*, 2016). PDGF-BB has been successfully incorporated in the core of PCL and PCL/polyethylene glycol (PEG) fibres (Liao *et al.*, 2006). Incorporation of PEG in the PCL shell through blending, rendered the fibres permeable to protein by inducing swelling and pore formation. The release kinetics could be controlled by varying the nature and amount of PEG in the shell of the nanofibres. Absence of PEG in the shell layer resulted in negligible release of PDGF-BB since PDGF-BB itself cannot generate open pores throughout the shell layer. A very small amount is in the core of the fibres and the diffusion through the bulk PCL shell could be too slow to take place in the desired time frame (Jiang *et al.*, 2014). In the different scaffolds tested, PDGF-BB exhibited sustained release profile over 35 d and it was shown to be bioactive (Liao *et al.*, 2006). Li and co-workers have produced dextran (DEX)/poly(L-lactide-co-epsilon-caprolactone) (PLCL) coaxially electrospun fibres carrying PDGF-BB. In their studies, the electrospun scaffolds showed a burst release of PDGF-BB in the first 2 d, followed by a steadier release up to 28 d (Li *et al.*, 2010).

So far, for tendon repair applications, coaxial electrospun scaffolds have not been utilised for delivery of PDGF, neither *in vitro* nor *in vivo*, while emulsion electrospun scaffolds have been explored *in vitro*. These techniques might offer an advantage over the heparin-based delivery systems. One disadvantage of the heparin-based delivery systems lie in the preparation step. The conjugation of heparin to the polymeric scaffold requires the immersion and incubation of the device with all the necessary solutions, including the heparin solution, and in the next step the incubation with PDGF solution. This process allows for complete conjugation of the scaffold with heparin and subsequently PDGF. However, once applied at the tendon injury site, PDGF delivery is desired preferably towards the

tendon and not the surrounding tissue, where its diffusion can affect neighbouring cells and lead to undesired side effects, such as adhesion formation (Meier Buergisser *et al.*, 2014; Meier Buergisser and Buschmann, 2015). Because of this, layered scaffolds could be considered as an alternative, which might allow for more spatial selection for PDGF delivery primarily towards the tendon tissue.

Using electrospinning techniques, a physical separation between the bioactive layer of the scaffold and the surrounding tissue of a tendon can be achieved, with a directed, localised delivery of PDGF-BB. Recently, this approach was associated with the use of a double-layered DegraPol® tube that has a bioactive and non-bioactive layer, to be applied over ruptured and conventionally sutured tendon in order to promote tendon healing with PDGF-BB delivery (Evrova *et al.*, 2016).

PART B: Effects of PDGF-BB delivery *in vivo*

Impacts of PDGF-BB

Although *in vitro* release studies are necessary for the characterisation of a delivery device, *in vivo* experiments and assessments are absolutely needed to discover its real effects in the field of tendon injuries or any other application explored. The problem often confronted is that *in vitro* release kinetics may differ from *in vivo* release kinetics, once the delivery device comes in contact with tissues and body fluids, where it is exposed to additional degradation by enzymes. Enzymes that are present at inflamed wound sites and found in plasma, by oxidation or hydrolysis, can affect the degradation rate of the material and, thus, influence the release profile from the device. Also, sterilisation methods, as part of *in vivo* procedures and regulations can affect the material degradation (Savaris *et al.*, 2016) and bioactivity of the incorporated growth factors (Mainil-Varlet *et al.*, 1997; Moioli *et al.*, 2006). Furthermore, the stability and bioactivity of the released growth factor at the wound site can differ from the *in vitro* conditions.

Fibrin-based delivery device

In vitro, PDGF-BB has been shown to support proliferation and DNA synthesis, as described in detail in part A. Hence, accelerated wound healing is expected *in vivo*, which has been confirmed for a fibrin-based system. Moreover, PDGF-BB was shown to improve the gliding capacity, an aspect that can only be confirmed by *in vivo* experiments. This fibrin-based delivery system, where PDGF-BB is attached to heparin, which is itself electrostatically bound to a peptide acting as a bridge to fibrin (Fig. 5**B**) (Sakiyama-Elbert *et al.*, 2008), was tested in a canine flexor tendon model (Thomopoulos *et al.*, 2007) (Table 3). The intrasynovial flexor tendons of the forepaw were fully and transversely transected, sutured with an 8-strand suture and the defect was filled with the gel, acting as a delivery system. 100 ng of PDGF-BB were incorporated into the delivery device and tendons were analysed histologically 7 and 14 d postoperatively. Cell density, proliferation, total DNA levels, reducible collagen crosslink levels and type I collagen expression were assessed. A clear beneficial effect was observed in comparison to the control group, where the dogs received the same transection, pocketing and suture without fibrin gel application. Cell density, proliferation and type I collagen expression were increased in PDGF-BB-treated specimens at both time points, compared to the control. Also, reducible collagen crosslinks were significantly increased 7 d post-operatively, which indicates that the PDGF-BB-treated tendons demonstrated accelerated healing; reducible crosslinks increased when the remodelling phase was entered. In contrast to these positively influenced parameters, the collagen organisation and the inflammatory reaction were similar in both groups, thus not affected by PDGF-BB. Moreover, with an increased dosage of 500 ng of PDGF-BB, incorporated in the same delivery system, and increase in cell density, proliferation and type I collagen expression were detected 14 d post-operation and confirmed previous results (Thomopoulos *et al.*, 2009).

This fibrin-based PDGF-BB delivery system was also evaluated 3 weeks post-surgery, in terms of gliding capacity (range of motion) and biomechanical strength (Gelberman *et al.*, 2007). Interestingly, PDGF-BB-treated specimen exhibited a higher range of motion. The rotations of the proximal interphalangeal (PIP) joint and the distal interphalangeal (DIP) joint were assessed, based on the differences between the flexed and extended positions. The DIP and PIP ranges of motion (ROMs) were significantly higher in the PDGF-BB group (around doubled ROMs). Besides its mitogenic effects, there is some evidence that PDGF-BB stimulates the biosynthesis of hyaluronan, one of the most studied and applied anti-adhesives (Meier Buergisser and Buschmann, 2015) and an important lubricant of the intrasynovial fluid in healthy tendons. Biosynthesis of hyaluronan, as a result of PDGF-BB application, has been reported for tenocytes (Thomopoulos *et al.*, 2009), as well as for prostate smooth muscle cells (Pullen *et al.*, 2001), cardiomyocytes (Hellman *et al.*, 2010) and temporomandibular joint disc-derived cells (Hanaoka *et al.*, 2006). The application at the wound site of a sustainable, releasing, PDGF-BB-loaded device, not only accelerated wound healing 1 and 2 weeks post-surgery (Thomopoulos *et al.*, 2007), but also enhanced significantly the gliding of the tendons, as found 3 weeks post-intervention (Gelberman *et al.*, 2007). In a subsequent study, where PDGF-BB amount was increased five-fold (to 500 ng) and the time point of tendons extraction was increased to 6 weeks, Thomopolous and co-workers confirmed the higher range of motion in the PDGF group, compared to control (Thomopoulos *et al.*, 2009). Moreover, the increase in hyaluronic acid in PDGF group, compared

Table 3. Summary of *in vivo* experiments and outcomes after PDGF-BB administration, key: ↑ = increase, n.a. = not affected, ↓ = decrease

Model	PDGF-BB dosage	Mode of administration	Time post operation	Outcomes		
				Cellular	Adhesion	Biomechanics
Canine flexor tendon full transection (Thomopoulos *et al.*, 2007)	100 ng	Pocketing of fibrin-based PDGF-BB attached to heparin gel (Sakiyama-Elbert *et al.*, 2008)	1, 2 week	Cell density ↑ Proliferation ↑ Total DNA ↑ Reducible collagen crosslinks ↑ Collagen I ↑ Collagen organization n.a. Inflammation n.a.	Range of motion ↑	
(Gelberman *et al.*, 2007)	100 ng		3 week			Ultimate force n.a. Stiffness n.a. Strain at 20 N n.a.
(Thomopoulos *et al.*, 2009)	500 ng	Fibrin gel with heparin-bound PDGF-BB + PLGA meshes; layers inserted in longitudinal slits	6 week		Range of motion ↑	Ultimate force n.a. Stiffness n.a. Strain at 20 N n.a.
(Manning *et al.*, 2013)	250 ng		9 d	Cellularity ↓ Vascularity ↓ Inflammation ↑	Hyaluronic acid ↑	
Rat Achilles tendinopathy (Shah *et al.*, 2012)	1.02 µg 10.2 µg 102 µg	Single bolus injection	1, 3 week	Proliferation 1.02 µg n.a.; 10.2 µg ↑;102 µg ↑ Inflammation 1.02 µg n.a.; 10.2 µg n.a.;102 µg ↑	Not assessed	Ultimate force 1.02 µg n.a.; 10.2 µg ↑;102 µg n.a. Ultimate stress 1.02 µg ↑; 10.2 µg n.a.;102 µg n.a.
(Solchaga *et al.*, 2014)	3 µg 10 µg		1, 3 week	Proliferation 3 µg n.a.; 10 µg ↑ Inflammation 3 µg n.a.; 10µg n.a.		Ultimate force (times ↑) 1w 3w 3 µg 1.96 1.36 10 µg 1.22 1.92 Stiffness (times ↑) 3 µg 1.58 1.09 10 µg 1.03 2.00
Rat rotator cuff (Kovacevic *et al.*, 2015)	0.2 µg 2 µg 6 µg	Collagen I carrier (BioBlanket)	5 d, 4 week	Proliferation ↑ (dose-dependent) Vascularity ↑ (dose-dependent) Proteoglycan n.a.	Not assessed	Ultimate force n.a. Stiffness n.a.
Rat Achilles tendon (Suwalski *et al.*, 2010)		pDNA plasmid encoding PDGF-BB on silica nanoparticles in three longitudinal incisions	15 d, 6 week	Inflammation n.a.	Not assessed	Ultimate force ↑ Young's modulus n.a. Stiffness ↑
(Delalande *et al.*, 2015)			2 week			

to control, supported one of the authors' hypotheses, namely that PDGF-BB stimulated the production of hyaluronic acid (Thomopoulos *et al.*, 2009).

Although the fibrin-based delivery device (Sakiyama-Elbert *et al.*, 2008) had been shown to promote tendon healing (Thomopoulos *et al.*, 2007) and gliding capability, it did not enhance the biomechanical properties 3 weeks post-surgery, with peak forces, stiffness and strain at 20 N being very similar for PDGF-BB group and control group. The authors attributed this ineffectiveness to PDGF-BB dosage – the gel had been loaded with only 100 ng of PDGF-BB (Gelberman *et al.*, 2007). In a later study, although the loading amount of PDGF-BB in the fibrin-based matrix was increased to 500 ng and the time point of post-operative examination extended to 6 weeks, the biomechanical properties were still similar with and without PDGF-BB delivery (Thomopoulos *et al.*, 2009). Even though the sustained release of PDGF-BB from the corresponding matrix was detected *in vitro* over 10 d (Thomopoulos *et al.*, 2007), *in vivo* conditions may differ in many aspects from *in vitro* conditions, affecting fibrin degradation, as well as, PDGF-BB release kinetics and stability at the wound site. Hence, promising findings *in vitro* have ultimately to be confirmed *in vivo*.

Delivery device in combination with stem cells

Although the fibrin-based system with PDGF-BB attached to heparin (Sakiyama-Elbert *et al.*, 2008) allowed for an enhanced flexor tendon healing (Thomopoulos *et al.*, 2007; Thomopoulos *et al.*, 2009), the handling of the hydrogel, with its soft consistency, was difficult during surgical implantation. To overcome the rather difficult consistency, the hydrogel was layered with electrospun PLGA fibre meshes. A second potential improvement was the simultaneous seeding of adipose-derived stem cells (ASCs) (Manning *et al.*, 2013). An alternating layered scaffold was constructed with PDGF-BB and ASCs incorporated in the heparin-fibrin delivery layers and with layers made of PLGA (no PDGF-BB, no cells). After a full transection of canine flexor tendons, longitudinally-oriented horizontal slits were created in the centre of each tendon stump and the layered scaffold was implanted and fixed by suture at the repair site. It was reported that the release of PDGF-BB *in vitro* was 22 % on the first day and progressed steadily to 71 % by day 9. In addition, *in vivo*, at the repair site, the fluorescently-labelled cells were still viable after 9 d. Although the presence of ASCs might further enhance the flexor tendon healing, the layered scaffold was also implanted with PDGF-BB alone, incorporated in the fibrin-based layers (no ACSs). In terms of cellular response during early healing, it was found that the cellularity and vascularity in the cell-free scaffold with PDGF-BB were slightly decreased at day 9 post-operation, when compared to the repair-only group (only suture). In contrast, inflammatory cells, such as foreign body giant cells, poly-morphonuclear cells and monocytes, were slightly increased in the acellular PDGF-BB system (Manning *et al.*, 2013), suggesting a mild immune response towards the scaffold material (PLGA). Hence, although acceleration of cell proliferation by PDGF-BB has been shown to be manifold *in vitro*, this example shows that total cell densities during the healing process change with time and, at certain time points after injury, may be decreased, compared to native densities – which is impossible to demonstrate *in vitro*. Moreover, , *in vivo*, at the repair site, the impact of PDGF-BB on inflammatory cells and the relative abundance of macrophages, monocytes, foreign body giant cells may differ from single cell cultures,, where the chemotactic effect of PDGF-BB on macrophages (Inaba *et al.*, 1993), monocytes and neutrophils (Deuel *et al.*, 1982) can be observed with a single focus on these cell types – regardless of the multiple orchestra of factors and cytokines released from all cell types present at the wound site. Again, we conclude that *in vivo* experiments are absolutely necessary to elucidate the mentioned issues.

Single bolus injection

As different enzymes may affect the stability and bioactivity of PDGF-BB *in vivo*, the outcomes for dosage's effect must be examined concisely. In a rat Achilles tendon model, it has been shown that lower dosages may positively affect biomechanical outcomes äat early time points, *i.e.* one week post-operation, while higher dosages have this effect only at later time points, *i.e.* 3 weeks. In this rat Achilles tendon model, tendinopathy was induced by collagenase, 7 d prior to PDGF-BB administration. When the healing tissue was analysed histologically for cellularity, collagen fibre orientation and density, inflammation and vascularisation, it was found that different amounts of single bolus applied at the wound site had different dose-dependent effects. Furthermore, biomechanical strength, 1 and 3 weeks post-administration, was also influenced by PDGF-BB (Shah *et al.*, 2013).

When doses of 1.02, 10.2 and 102 μg of PDGF were applied, the cell proliferation was significantly increased at 10.2 and 102 μg, but not at 1.02 μg of PDGF. 3 weeks post-application, inflammatory reaction and vascularisation were significantly increased only at the highest dose (102 μg of PDGF). Furthermore, assessment of biomechanics revealed that only the highest dose group had significantly larger failure loads at 1 and 3 weeks, compared to the other treated groups – indicating a biphasic dose-dependence and the need for an exact evaluation of the optimum growth factor amount (Shah *et al.*, 2013).

Solchaga *et al.* (2014) also worked with a single injection of PDGF-BB. Either 3 or 10 μg of PDGF-BB, dissolved in 30 μL of PBS, were intra-tendon injected in a rat Achilles tendon model, where the tendinopathy was induced by collagenase. The proliferating cells were quantified in a histological section by proliferating cell nuclear antigen (PCNA) positive cell counting. It was found that cell proliferation was

significantly increased, with 65 % more proliferating cells, in the 10 µg of PDGF-BB-group, compared to saline control. In terms of inflammatory reaction, no different effect was detected for both doses, when compared to the control.

In this tendinopathy model, biomechanical properties of the tendons were positively influenced 1 and 3 weeks post-administration and only at 10.2 µg of PDGF. Other concentrations have not shown a significant difference compared to the normal untreated control (Shah *et al.*, 2013). On the other hand, ultimate tensile stress was only significantly higher, compared to the other groups, in the 1.02 µg of PDGF group. Such findings stress the importance of an appropriate dosage, which can be easily and exactly chosen when single bolus injections are used, but is a more delicate and difficult issue when growth factor delivery devices are used to release the factor in a controlled way. The beneficial effects in terms of biomechanics were reported also for a rat Achilles tendon tendinopathy model, where 3 or 10 µg of PDGF-BB were applied intra-tendon as a single injection (Solchaga *et al.*, 2014). Analysis post-surgery revealed that the ultimate loads were increased by factor of 1.96 and 1.36 for the 3 µg group and 1.22 and 1.92 for the 10 µg group all at 7 and 21 d post-surgery, respectively. Similarly, stiffness increased by factors of 1.58 and 1.09 for the 3 µg group and 1.03 and 2.00 for the 10 µg group at 7 and 21 d, respectively. It may be concluded that smaller amounts of PDGF-BB, such as 3 µg, enhance biomechanics in the first week, while larger amounts of PDGF-BB, such as 10 µg, lead to better biomechanical outcomes only later (as shown here for 3 weeks) (Solchaga *et al.*, 2014).

PDGF-BB adsorbed on collagen

In a rat rotator cuff model, PDGF-BB was applied in three different dosages using a commercial collagen I scaffold (BioBlanket Surgical Mesh; Kensey Nash, Exton, PA, USA). Either 0.6, 2 or 6 µg of PDGF-BB were dissolved in a sodium acetate buffer and small volumes of it were adsorbed on the scaffold immediately before surgical implantation (Kovacevic *et al.*, 2015). Histological and biomechanical analyses were performed at 5 and 28 d, respectively. Cellular proliferation, detected with PCNA immunohistochemical analysis at day 5, was found to be dose-dependent, with the highest number of actively dividing cells at the highest dosage of PDGF-BB. The same was found for the angiogenic response: the strongest staining was detected for the highest dosage of PDGF-BB, when analysed with Factor VIII immunohistochemistry. However, proteoglycan staining and collagen birefringence, both analysed 28 d post-surgery, did not reveal any difference between the PDGF-dosage groups, nor between the groups and the control, where a collagen scaffold without PDGF-BB was implanted (Kovacevic *et al.*, 2015).

Although promising effects at the cellular level were found for this collagen delivery system, with PDGF-BB adsorbed in different dosages, no beneficial effect were observed 28 d post-operation on the biomechanical outcome, when the delivery system was applied as a rat supraspinatus augmentation (Kovacevic *et al.*, 2015). No significant differences were measured in ultimate load and stiffness, when the scaffolds loaded with 0.6, 2.00 or 6 µg of PDGF-BB were compared to the pure scaffold.

Non-viral PDGF-BB gene transfer

Surface-modified silica nanoparticles (functionalisation with amino- and subsequently carboxylic-functional groups) were used as a vehicle for pDNA plasmid encoding PDGF-B gene (Suwalski *et al.*, 2010). In a rat Achilles tendon model, three longitudinal incisions were made and 50 µL of the pDNA/nanoparticle (2 : 5) were injected into the middle incision. 15 d post-treatment, biomechanical measurements revealed that the gene transfer using these nanoparticles improved the ultimate load, while the Young's modulus was similar, when compared to the control. As silica may induce inflammatory reaction when used as an implant material (Lin *et al.*, 2006), Suwalski and co-workers investigated this aspect as well. Histological analysis revealed no local inflammation nor necrosis, even when analysed after 6 weeks (Suwalski *et al.*, 2010).

Another type of non-viral-based gene transfer vehicle, realised with liposomes, was developed and beneficially applied in a rat Achilles tendon model with triple longitudinal incisions used as tendon laceration. 50 µL of liposomes, of two different compositions, containing 20 µg of plasmids DNA encoding PDGF gene were slowly injected into the middle section of the Achilles tendon. 14 d post-injection, the PDGF group showed a stiffness of 83 % compared to uninjured tendon, while the non-treated control only reached 72 %, indicating a beneficial *in vivo* effect at early time points during the healing process (Delalande *et al.*, 2015).

PDGF-BB coated sutures

As an alternative to the delivery systems described above, Cummings *et al.* (2012) reported on a PDGF-BB-coated Vicryl suture, applied in a rat Achilles tendon transection model. By dipping the sutures in either 0 (control), 0.3, 3 or 10 mg × mL^{-1} of PDGF solutions, the authors could show the beneficial effect, 4 weeks post-operatively, of PDGF-BB-coated suture. The biomechanical properties increased in a dose-dependent manner and improved tissue remodelling with decreased tendon cross-sectional area and improved collagen organisation in the repaired tendons.

Current limitations and future work

PDGF-BB is well known for its mitotic, chemotactic and angiogenic activity (Anitua *et al.*, 2012) and because of this, it is considered as a promising

candidate for tendon repair. As it is FDA approved and used in clinical settings for other applications, no safety problems are expected to arise with its use for tendon repair (Paul *et al.*, 2015; Solchaga *et al.*, 2012; Ziyadeh *et al.*, 2011). Many *in vitro* studies with tenocytes clearly demonstrate its biological properties (Raghavan *et al.*, 2012; Zhao and Hadjiargyrou, 2011), while studies of its effects on the tendon progenitor stem cell population are still missing. On the other hand, most studies explored cell responses in 2D environment, while this can differ in 3D models (Antoni *et al.*, 2015; Pampaloni *et al.*, 2007) and can help bridge the gap with *in vivo* experimental outcomes. Even though there are some observations, systematic information is still missing on how PDGF-BB alone, or in interplay with other growth factors, could affect specific tendon markers (tenomodulin, Scx, Mohawk *etc.*).

So far, *in vitro* experiments do not allow for direct correlations and conclusions when it comes to prediction of *in vivo* outcomes. *In vivo* models are necessary for determining its biological impact on biomechanical properties (Buschmann and Meier Buergisser, 2017) or tissue composition. Similarly, the effectiveness of any PDGF-BB delivery device, allowing for its sustained delivery over a longer period in *in vitro* conditions, accompanied with promising outcomes, can differ *in vivo*, potentially resulting in reduced effectiveness in aiding tendon repair. Two main parameters for *in vivo* effectiveness are dosage and time of application, which may be difficult to simulate in cell culture conditions or in three-dimensional cultures using tissue engineered constructs (Juncosa-Melvin *et al.*, 2006). Low dosages (~ 100 ng) of PDGF-BB delivered *in vivo* in a sustained manner enhanced cell proliferation, but did not show any beneficial impact on biomechanics (Thomopoulos *et al.*, 2007). Only higher doses (~ 500 ng) of PDGF-BB delivered in the same way resulted in beneficial effects (Thomopoulos *et al.*, 2009). On the other hand, when single bolus injections (Shah *et al.*, 2013) or different scaffolds/carriers are used for delivery (Kovacevic *et al.*, 2015), much larger amounts (1-10 μg) might be needed to have any effect on the biomechanics of the tendons. With the current studies suggesting higher dosages of PDGF-BB as promising, a systematic study in one *in vivo* model, with different delivery methods and dosages and the possibility to track the release profile of the growth factor *in vivo* is missing. These types of studies would offer valuable insights into the mechanism of release and what really plays an important role in improving the resulting biomechanical properties.

The proper time of application and delivery is the second important aspect that can affect the success of the biological therapy with PDGF-BB. Very early administration (immediate up to 3 d post-injury) in a single shot did not add any benefits to the healing process and might not be the correct approach. Ideally, PDGF-BB should be applied around 1 week post-laceration (Chan *et al.*, 2006; Gulotta and Rodeo, 2009) by a single bolus injection. Even better, it could be incorporated in a delivery system that allows its sustained release to the wound site over the duration of at least two weeks. Delivery systems that partially degrade during the first week post-operation, leading to largest release of PDGF-BB after this period, could be considered as promising candidates for tendon rupture repair. However, careful and comprehensive degradation and integration studies of the delivery device *in vivo*, without any growth, factor should be initially performed. For example, the polymer DegraPol®, intended to be used as a PDGF-BB delivery device for tendon repair (Evrova *et al.*, 2016), has been thoroughly investigated for its *in vivo* effects in terms of cellular response prior to PDGF-BB incorporation (Buschmann *et al.*, 2013; Buschmann *et al.*, 2015). Moreover, there is little to no knowledge about the half-life of delivered PDGF-BB at the wound site from the different devices or its half-life within the different biomaterials and devices. This can be an important parameter to be determined for establishing the effectiveness of any proposed delivery strategy and needs future research. Future studies should focus on standardised animal models to enable the comparison of different delivery devices, at best implanted in the same way into the same animal model. Moreover, methods, readouts and time points of post-operative analysis should be standardised to allow for a full and adequate comparison of different delivery strategies, dosages used or time points tested. Only data resulting from comparable *in vivo* experiments could be used to elucidate which strategy might be best to be transferred into clinical trials later. Unfortunately, so far, different research groups have focused on selected aspects, such as only biomechanical readouts, only inflammation or tissue remodelling *etc.*, which renders the puzzle difficult to be fully solved.

Another focus of future work should be gaining more insight into mechanisms that might play a role in improved biomechanics or tissue organisation. A closer look at tissue remodelling at the wound site, what extracellular matrix components are present, as well as what tendon specific markers might be upregulated or downregulated during the treatment period with PDGF-BB, may offer knowledge as to how the healing might be affected and in turn how this can be used for better design of delivery devices or optimisation of application time.

Conclusion

Biological therapies after tendon ruptures or lacerations have many faces. Acceleration of the healing process with a true regeneration of the tendon tissue is a predominant desired aim. All cues leading to a faster cell infiltration to the wound site, with increased cell proliferation and accompanied with proper ECM remodelling are welcomed (Elliot and Giesen, 2013a; Elliot and Giesen, 2013b). Thus, the use

of growth factors delivered at the injury site, either single or applied as a cocktail of many (Nourissat *et al.*, 2013), provides a promising approach to support the tendon healing process. Different growth factors including TGFβ1, PDGF, VEGF and fibroblast growth factor-2 (FGF-2) have been explored for tendon repair and have shown mixed responses of the regenerated tendons in terms of biomechanical and histological outcomes. VEGF was shown to have deleterious effect on tendon healing due to MMP-3-supported angiogenesis, with inferior biomechanical properties of the tendons (Sahin *et al.*, 2012). Local delivery of TGFβ1 was shown to improve the biomechanical and histological properties of the tendons (Halper, 2014; Majewski *et al.*, 2012). However, TGFβ1 is a growth factor associated with complex biological signalling and scar and adhesion formation (Chang *et al.*, 2000b; Galatz *et al.*, 2006; Penn *et al.*, 2012). Exogenous delivery of FGF-2 in a canine model did not result in improved biomechanical or molecular properties of the treated tendons (Thomopoulos *et al.*, 2010b), on the other hand FGF-2 gene transfer has yielded more promising results (Tang *et al.*, 2008). In comparison, and as summarised in this review, PDGF-BB with its clinical approval and biological properties represents a safe and promising growth factor to be applied as biological therapy in tendon rupture repair, administered either by bolus injection or incorporated in an implantable delivery device.

From the literature covered in this review and the observations existing so far, it can be concluded that from the available delivery strategies, a single bolus injection of 10 μg of PDGF-BB one week post-operation (Shah *et al.*, 2013) or a fibrin-based PDGF-BB delivery system loaded with much larger amounts than reported, *e.g.* 10 μg instead of only 0.5 μg (Thomopoulos *et al.*, 2009), would probably be the most promising approaches. However, for its potential to be fully exploited, crucial aspects like dosage, time of application and delivery method need to be carefully considered, chosen and further investigated in one and same *in vivo* model, while looking at many readouts as possible including biomechanics, inflammation and tissue remodelling.

Disclosure

The authors confirm that there are no conflicts of interest.

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Discussion with Reviewer

Denitsa Docheva: How do the authors foresee the integration of PDGF-BB delivery devices, such as electrospinned polymers, once the healing process has been activated and the initial granulation tissue has been formed?

Authors: The way of PDGF-BB application one week after the operation is not meant to be performed by reopening the wound and manipulating for a second time. In contrast, delivery devices releasing the largest amount of PDGF-BB one week after the operation are desired systems. Partial degradation of the delivery device after the initial week should lead to highest PDGF-BB release at that point, as mentioned in "Current limitations and future work" paragraph.

On the other hand, the *in vivo* performance of the delivery device, *e.g.* electrospun scaffolds, should be considered at the starting point, even before incorporating a growth factor. Careful and comprehensive degradation and integration studies of the devices must precede any further investigation. Cell infiltration and biomaterial encapsulation is how the integration of the electrospun scaffolds usually takes place and with time, if biodegradable, the material is degraded and reabsorbed.

Our research group has used the polymer DegraPol® as a drug delivery device. As a first step, we investigated the effects in terms of *in vivo* cellular response towards this foreign material and focused on its integration. We found that it was very well accepted and no further inflammatory response was found. Moreover, *in vivo*, after 12 weeks , the degradation products were well integrated and penetrated by invading cells (Buschmann *et al*., 2013; Buschmann *et al*., 2015).

Editor's note: The Scientific Editor responsible for this paper was Juerg Gasser.